Pulse Photoplethysmographic Analysis Estimates the Sympathetic Activity Directed to Heart and Vessels

Riccardo Colombo, M.D., Andrea Marchi, M.Eng., Beatrice Borghi, M.D., Tommaso Fossali, M.D., Roberto Rech, M.D., Antonio Castelli, M.D., Alberto Corona, M.D., M.Sc., Stefano Guzzetti, M.D., M.Sc., Ferdinando Raimondi, M.D.

ABSTRACT

Background: Novel pulse photoplethysmographic–derived indices have been proposed as tools to measure autonomic nervous system (ANS) modulation in anesthetized and awake patients, but nowadays their experimental validation is lacking. The authors aimed to investigate the ability of pulse photoplethysmographic amplitude (PPGA), ANS state (ANSS), and ANSS index (ANSSi) to measure changes of ANS modulation in response to sympathetic stimulation.

Methods: Ten awake healthy volunteers underwent two passive head-up tilts at 45° and 90°. The heart rate variability (HRV) and systolic arterial pressure variability were analyzed in the frequency domain as a measure of ANS modulation directed to the heart and the vessels. HRV, baroreflex sensitivity, and pulse photoplethysmographic indices were measured at baseline and after tilt maneuvers. The agreement between HRV-derived indices and pulse photoplethysmographic indices was assessed using Bland–Altman plots.

Results: PPGA, ANSS, and ANSSi changed significantly during the study protocol. Head-up tilt decreased PPGA and ANSS and increased ANNSi. There was a good agreement between ANSSi and baroreflex sensitivity explored in the high-frequency band (bias, 0.23; 95% CI, −22.7 to 23.2 normalized units) and between ANSSi and the sympathovagal modulation directed to the heart (bias, 0.96; 95% CI, −8.7 to 10.8 normalized units).

Conclusions: In controlled experimental conditions, novel pulse plethysmographic indices seem to estimate the changes of the sympathetic outflow directed to the vessels and the sympathovagal balance modulating heart rate. These indices might be useful in the future to monitor the fluctuation of sympathetic activity in anesthetized patients.
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THE autonomic nervous system (ANS) modulates the heart beat-to-beat interval and peripheral muscle vascular tone in response to a multitude of stimuli. It is known that surgical stimulation induces a stress response[1,2] characterized by sympathetic activation increasing heart rate, blood pressure, catecholamine, and corticosteroid release, and it has been supposed influencing postoperative outcome.[3,4]

Measuring changes of ANS activity during general anesthesia is an exciting challenge for the anesthesiologists. In a daily clinical practice, titrating the depth of hypnosis and analgesia to maintain a stable level of sympathetic activity might exert interesting effects on patients' outcome. Unfortunately, the direct measure of the sympathetic and vagal activity seems not feasible in a clinical setting. Traditionally, indirect measurement of the ANS modulation on cardiovascular system was based on the analysis of the heart rate variability (HRV) and systolic arterial pressure (SAP) variability in well-established experimental conditions.[5,6] This type of analysis is still confined in a research setting because of complexity of interplay of cardiovascular signals and it does not seem to provide a clear and simple parameter to inform the anesthesiologist in real time about ANS activity. Recently, some authors stated that two indices derived from

What We Already Know about This Topic

- Surgical stimulation induces a stress response characterized by sympathetic activation increasing heart rate, blood pressure, catecholamine, and corticosteroid release
- This study determined the ability of pulse photoplethysmographic amplitude, autonomic nervous system state, and autonomic nervous system state index to measure changes of autonomic nervous system modulation in response to a gravitational challenge in healthy adults

What This Article Tells Us That Is New

- In controlled experimental conditions, novel pulse plethysmograhic indices estimated changes of the sympathetic outflow directed to vessels and the sympathovagal balance modulating heart rate

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pulse photoplethysmographic amplitude (PPGA) analysis of the photoplethysmograph, namely ANS state (ANSS) and ANSS index (ANSSi), would be useful tools to measure the autonomic modulation.[7,8] In fact PPGA is because of pulsatile changes in tissue volume, mainly arterial blood, and it decreases during sympathetic mediated vasoconstriction. Unfortunately, until today, the experimental validation of ANSS and ANSSi was never been provided. The aim of this study is to assess the accuracy of ANSS and ANSSi to measure changes in ANS modulation directed to the heart and the vessels in response to a sympathetic stimulus, elicited through a gravitational load, in healthy adult humans.

Materials and Methods

After the approval of local institutional ethical committee (Luigi Sacco Hospital, Milan, Italy), we studied the effects of two orthostatic challenges on HRV, SAP variability, PPGA, ANSS, and ANSSi in 10 awake adult volunteers. The study adhered to the principles of the Declaration of Helsinki for medical research involving human subjects, and written informed consent was obtained from all volunteers.

All subjects were healthy, did not take any drug, beverages containing caffeine or alcohol during the previous 24 h, and were asked to fast 3 h before the study protocol. Electrocardiogram and photoplethysmographic waves were recorded through a S/5 Avance monitor (GE, Finland) to a laptop computer provided with S/5 Collect software (GE), and they were sampled at 300 Hz. The photoplethysmograph probe was a standard pulse oximetry probe in endowment to the commercial S/5 Avance monitor and was positioned on the third finger of the right hand. Noninvasive continuous arterial pressures were recorded by Nexfin (Edwards Lifesciences, USA) through a cuff positioned on the third finger's middle phalanx of the right hand, sampled at 400 Hz, and stored to a laptop computer running LabChart Pro 7 (ADInstruments, New Zealand). All signals were synchronized at the beginning of the recording and analyzed offline. During the study sequence, the healthy volunteers were asked to lie calmly, breathing in rhythm with a metronome at 18 breaths per minutes (0.3 Hz), and they were not allowed to talk.

It is well known that changes in position of patients can lead to changes in the balance of the ANS. One of the most accepted maneuver to stimulate ANS-mediated cardiovascular control is head-up tilt (HUT).[9–13] Tilt causes shift of blood toward lower body and reduction of the venous return. These effects induce a compensatory baroreflex-mediated increase of heart rate and peripheral vascular resistance aiming to maintain arterial pressure near to the level preceding the challenge.[13] The experiment protocol consisted of a sequence of passive postural changes, each of them lasting 10 min. The sequence was (1) baseline recording in supine position (bas), (2) HUT at 45° (HUT45), (3) recovery after HUT45, (4) HUT at 90° (HUT90), and (5) recovery after HUT90. The postural changes were gained with a tilt table (TILT TEST®, Gardhen Bilance, Italy) present in our institution.

Measurements and Extraction of Beat-to-Beat Variability Series, ANSS, and ANSSi

After detecting the QRS complex on the electrocardiographic wave and locating the R-apex using parabolic interpolation, the temporal distance between two consecutive R parabolic apexes was computed and used as an approximation of the heart period. The maximum of arterial pressure inside heart period was taken as SAP. The occurrences of QRS and SAP peaks were manually checked to avoid erroneous detections or missed beats. If isolated ectopic beats affected the heart period and SAP values, these measures were linearly interpolated using the closest values unaffected by ectopic beats. Heart period and SAP measures were performed on a beat-to-beat basis. The sequences of 300 values after 2 min from changing position were selected inside each experimental step. The mean (μ) and the variance (σ^2) of heart period and SAP are expressed in ms, mmHg, ms^2, and mmHg^2, respectively.

The maximum pulse plethysmographic amplitude within each heart period was detected on the photoplethysmographic wave (fig. 1). Pulse-to-pulse interval was detected as interval between consecutive pulse photoplethysmographic peaks. ANSS was calculated as a mean of ANSS of 300 consecutive pulse beats, corresponding to beat-to-beat interval analyzed from electrocardiogram, and ANSSi was calculated from the same series following the equations[7,8]:

$$\mathrm{ANSS} = \mathrm{PPI}(\mathrm{ms}) \times \mathrm{PPGA}(\%) \quad (1)$$

$$\mathrm{ANSSi} = 100 - \left(\frac{\mathrm{ANSS}}{\mathrm{ANSS}_{\max}} \right) \times 90 \quad (2)$$

where PPI is the pulse-to-pulse interval of the photoplethysmogram, PPGA is the pulse PPGA, and $\mathrm{ANSS}_{\max}$ is the largest ANSS for the subject among 300 beats during each experimental condition.

Power Spectral Analysis

The power spectrum was estimated according to an univariate parametric approach fitting the series according to an autoregressive model.[9] Autoregressive spectral density was factorized into components, each of them characterized by a central frequency. A spectral component was labeled as low frequency (LF) if its central frequency was between 0.04 and 0.15 Hz, whereas it was classified as high frequency (HF) if its central frequency was between 0.15 and 0.5 Hz.[6] The LF and HF powers were defined as the sum of the powers of all LF and HF spectral components, respectively. The HF spectral density of heart beat intervals ($\mathrm{HF}_{\mathrm{RR}}$), expressed in absolute units (ms^2), was used as a marker of vagal modulation directed to the heart,[5] whereas the LF spectral density of systolic arterial pressure oscillations ($\mathrm{LF}_{\mathrm{SAP}}$), expressed in absolute units (mmHg^2) was used as a marker of sympathetic

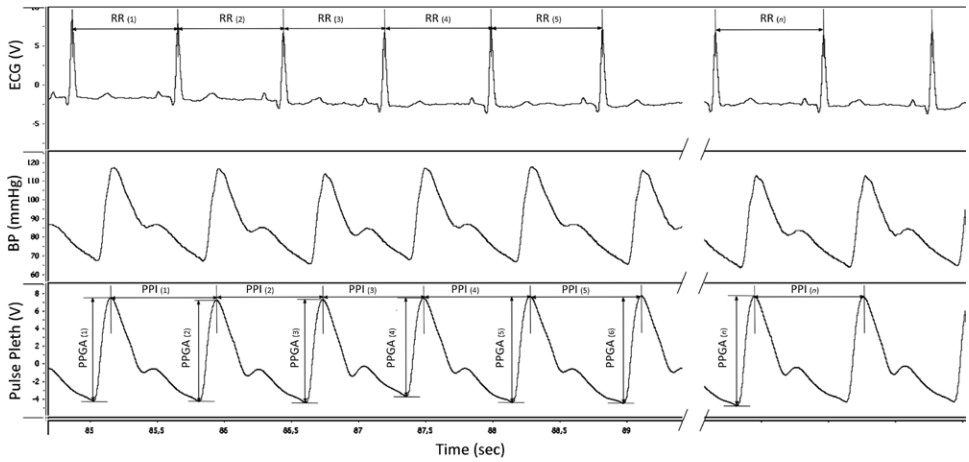

Fig. 1. Sample of recorded waves from a healthy subject. BP = noninvasive continuous blood pressure wave; ECG = electrocardiographic wave; PPGA = pulse plethysmographic amplitude; PPI = pulse-to-pulse interval on photoplethysmographic wave; Pulse Pleth = photoplethysmographic wave; RR = heart period.

modulation directed to the vessels.[14] The ratio of the LF_{RR} power to the HF_{RR} (LF_{RR}/HF_{RR}) was considered an indicator of the balance between sympathetic and vagal modulation directed to the heart (sympathovagal balance).[10] When LF_{RR}/HF_{RR} increases, it indicates the predominance of sympathetic over vagal activity. The power of heart period and SAP series in LF and HF bands was calculated as well and utilized for the estimation of the baroreflex sensitivity (BRS) (equations 3 and 4).

BRS Assessment

BRS was assessed according to a spectral analysis.[15] This method was grounded on the evaluation of LF_{RR}, LF_{SAP}, HF_{RR}, and HF spectral density of systolic arterial pressure oscillations (HF_{SAP}). BRS was computed as follows:

$$\alpha_{LF} = \sqrt{\frac{LF_{RR}}{LF_{SAP}}} \quad (3)$$

$$\alpha_{HF} = \sqrt{\frac{HF_{RR}}{HF_{SAP}}} \quad (4)$$

and expressed in ms·mmHg^{-1}. The prerequisites of high correlation (*i.e.*, >0.5) and negative phase between heart period and SAP series, indicating that heart period changes lagged behind SAP variations, were tested according to the calculation of squared coherence ($K^2_{RR,SAP}$) and phase spectrum ($Ph^2_{RR\text{-}SAP}$).[16] The cross-spectrum was estimated with a bivariate parametric approach fitting the heart period and SAP series according to a bivariate autoregressive model.[17]

Statistics

Sample size was calculated with PS Power and Sample Size Calculator Software for Windows (Vanderbilt University, U.S.A.).[18] In a previous study, ANSSi increased from 23.3 (SD, 13.7) to 78.1 (SD, 7.4) in response to painful stimulus.[7] We needed to study seven subjects to detect a mean ANSSi difference of 30 (SD, 15) between baseline and HUT90 with a power of 0.8 and α error of 0.05. However, we planned to enroll 10 subjects in the eventuality of artifacts precluding HRV analysis. For continuous variables, we used mean (SD) or median (interquartile range) when not normally distributed. The normality distribution was checked using the Kolmogorov–Smirnov test. We performed the one-way ANOVA, preceded by Levene's test, for repeated measures and *post hoc* Bonferroni test for multiple comparison. The correlation between HRV variables and pulse photopletysmographic–derived indices were checked with the Spearman correlation coefficient. With the hypothesis assessed by some authors[7,8] that pulse photoplethysmographic–derived indices measure ANS activity, we compared HRV variables and pulse photoplethysmographic variables through Bland–Altman plot.[19] Because the studied variables have different measuring units, we normalized each data series to a common scale to test their agreement on measuring ANS activity. Normalization consisted of 0 to 1 normalization following the formula $X' = \frac{X - X_{min}}{X_{max} - X_{min}}$ and multiplying the obtained value per 100, where X was a value of the series, and X_{min} and X_{max}, respectively, were the lowest and the highest value of the series. We proceeded to the normalization of our measures to limit the excursion of a set of values within a certain predefined range. However, normalization—as it is a mathematical procedure—cannot correct error in measuring; moreover, our measures were determined using interval level of measurement. Because we recorded data at five time points for all patients, we performed the Bland–Altman analysis adjusted for a random effect model to estimate within-subject variance, in which each subject has a different intercept and slope over the observation period.[20] In this analysis, we entered the "subject" and the "time point" (without interaction term) into the model as random effects.

As fixed effects, we entered "position" (supine or upright) and the "mean of values" of 10 subjects at each time point. Statistical analysis was carried out using GraphPad Prism 5 (GraphPad Software Inc., USA) and R 3.0 with *lme4* package for the mixed model analysis. For all test, *P* value less than 0.05 was considered as significant.

Results

All subjects completed the study protocol, and none of them experienced symptoms of hypotension or syncope. Results of univariate analysis of heart period and SAP series from 10 healthy volunteers (median age, 27.5 yr; range, 25 to 34 yr; M/F = 4/6) are summarized in table 1 and fig. 2. Mean heart rate increased during head-up position respect to the supine position, whereas SAP remained unchanged. The indices derived from the analysis of heart period and SAP in the frequency domain (HF_{RR}, LF_{RR}/HF_{RR}, and LF_{SAP}) showed a significant variability during the study protocol. LF_{SAP} increased significantly from baseline to HUT45 and HUT90 and LF_{RR}/HF_{RR} increased significantly at HUT 90, whereas HF_{RR} decreased significantly from baseline to HUT90.

All measurement of BRS satisfied the prerequisite of squared coherence ($K^2_{RR,SAP}$) and phase spectrum ($Ph^2_{RR\text{-}SAP}$).[16] The baroreflex sensitivity in the HF band (αHF) decreased significantly from baseline (14.9 ms·mmHg^{-1} [11.7 to 20.4 ms·mmHg^{-1}]) to HUT45 (7.6 ms·mmHg^{-1} [5.8 to 11.6 ms·mmHg^{-1}], $P < 0.05$) and HUT90 (4.8 ms·mmHg^{-1} [2.4 to 6.3 ms·mmHg^{-1}], $P < 0.05$).

All pulse photoplethysmographic–derived indices (PPGA, ANSS, and ANSSi) changed significantly during the study protocol (one-way ANOVA, $P < 0.0001$). PPGA decreased from baseline (4.4% [1.9 to 11%]) to HUT45 (2.9% [1.7 to 3.7%], $P < 0.05$) and HUT90 (1.8% [0.9 to 2.6%], $P < 0.05$) (fig. 2B). ANSS decreased from baseline (3.7% [2 to 7.4%], $P < 0.05$) to HUT45 (2.6% [1.5 to 3.2%], $P < 0.05$) and HUT90 (1.4% [0.6 to 2%], $P < 0.05$) (fig. 2C). ANSSi increased from baseline (64.9 [37 to 80.3]) to HUT45 (78.3 [73.7 to 83], $P < 0.05$) and HUT90 (87 [84.6 to 89.9], $P < 0.05$) (fig. 2D). There were not significant differences of PPGA, ANSS, and ANSSi between HUT45 and HUT90. Additional data are shown in the appendices 1 and 2.

There were significant correlations between crude values of αHF and pulse photoplethysmographic indices and between the sympathovagal balance (LF_{RR}/HF_{RR}) and ANSSi (table 2). After normalization of the series and the application of the linear mixed model (correcting for within-subjects variance), the resulting intercepts of ANS variables of all subjects are compared through Bland–Altman plots (fig. 3). There was good agreement between (1) αHF and ANSSi (bias, 0.23 normalized units [NU]; 95% CI, −22.7 to 23.2 NU) and (2) LF/HF and ANSSi (bias, 0.96 NU; 95% CI, −8.7 to 10.8 NU) in measuring ANS modulation during the study protocol.

Discussion

In 10 awake healthy subjects, we found that during gravitational sympathetic stimulation, as expected, SAP power in the LF band increased, baroreflex sensitivity assessed in the HF band decreased, and sympathovagal balance increased toward a prevalence of sympathetic modulation over vagal activity. Moreover, the HF power of HRV was reduced during tilt. These findings suggest a significant increase of sympathetic modulation directed to the vessels and a reduction of vagal modulation directed to the heart. In the orthostatic position, the blood shift to the lower body determines the decrease in central blood volume and pressure unloading cardiopulmonary and carotid baroreceptors, thus leading to tachycardia, splanchnic, and cutaneous vasoconstriction.[21–23]

Table 1. Heart Rate and Systolic Arterial Pressure Variability Characteristics of the Studied Subjects

	Baseline	HUT45	R1	HUT90	R2	*P* Value (ANOVA)
μ_{RR} (ms)	863 (800–1,066)	838 (800–926)*	990 (937–1,121)	746 (691–805)*†	984 (930–1,127)	<0.0001
σ^2_{RR} (ms^2)	256 (173–343)	258 (132–504)	348 (260–503)	221 (116–362)	265 (189–585)	0.064
HF_{RR} (ms^2)	94 (32–172)	26 (18–78)	121 (46–194)	14 (7–66)*	96 (51–340)	0.025
LF_{RR}/HF_{RR}	1.08 (0.56–1.63)	1.86 (0.72–6)	1.04 (0.65–2.13)	4.66 (2.9–8.5)*	0.99 (0.63–1.37)	0.013
μ_{SAP} (mmHg)	127 (123–133)	126 (119–131)	126 (123–138)	128 (122–138)	124 (117–136)	0.64
σ^2_{SAP} (mmHg^2)	30.2 (21.5–42.4)	57.2 (21.2–63.7)	66 (29.8–78.5)	55.3 (30.7–67.6)	40.6 (21.9–54.7)	0.009
LF_{SAP} (mmHg^2)	2.5 (0.7–6.4)	14.2 (5.2–19.6)*	4.4 (1.4–9.6)	15.8 (8.5–21.4)*	5 (1.5–7.2)	<0.0001
αHF (ms/mmHg)	14.9 (11.7–20.4)	7.6 (5.7–11.6)*	19.4 (16.1–26.1)	4.8 (2.4–6.3)*	19.5 (15.8–32.2)	<0.0001
αLF (ms/mmHg)	9.4 (4.7–16.7)	9.1 (5–13.6)	13.9 (7.9–21.3)	7.2 (5.9–9.9)	16 (8.6–21.1)	0.11

Values collected from the studied subjects at five time points during the study protocol. Values are expressed as median (interquartile range). *P* values are assessed with one-way ANOVA. Significances between the study phases of μ_{RR} and αHF are simplified. In this table, only significance at HUT45 and HUT90 is reported. A more comprehensive significance of differences between all study phases is illustrated in appendix 2.

* $P < 0.05$ *vs.* baseline. † $P < 0.05$ *vs.* HUT45.

HF_{RR} = high-frequency spectral density of heart beat intervals; HUT45 = head-up tilt at 45°; HUT90 = head-up tilt at 90°; LF_{RR} = low-frequency spectral density of heart beat intervals; LF_{RR}/HF_{RR} = sympathovagal balance of the studied subjects; LF_{SAP} = low-frequency spectral density of systolic arterial pressure oscillations; R1 = recovery after HUT45; R2 = recovery after HUT90; αHF = baroreflex sensitivity in the high-frequency band; αLF = baroreflex sensitivity in the low-frequency band; μ_{RR} = mean of heart beat-to-beat intervals; μ_{SAP} = mean of systolic arterial pressure; σ^2_{RR} = variance of heart beat-to-beat intervals; σ^2_{SAP} = variance of systolic arterial pressure.

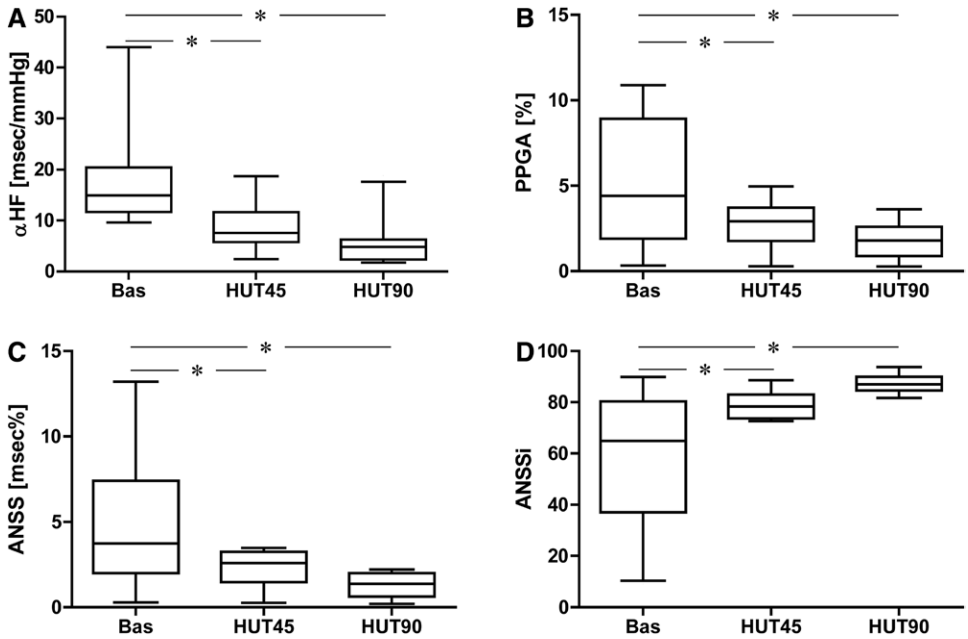

Fig. 2. The median (10th to 90th percentiles) of baroreflex sensitivity in the high-frequency band (αHF, *A*), pulse photoplethysmographic amplitude (PPGA, *B*), autonomic nervous system state (ANSS, *C*), and autonomic nervous system state index (ANSSi, *D*) during the study protocol. Data during recovery period are not shown. * A significant difference between *boxes* ($P < 0.05$) assessed with ANOVA for repeated measurements at five time points and Bonferroni *post hoc* test. Bas = baseline; HUT45 = head-up tilt at 45°; HUT90 = head-up tilt at 90°.

We found that pulse photoplethysmograph–derived indices changed significantly during tilt, thus suggesting that they reflect mainly the sympathetic-mediated vasoconstriction. It is well demonstrated that different types of sympathetic stimuli (painful, orthostatic, and lower body negative pressure) increase the firing rates of sympathetic muscular fibers resulting in vessels constriction.[11,24–26] These effects are the principal determinants of PPGA changes because of sympathetic stimulation. The pulse PPGA is because of pulsatile changes of the arteriolar blood volume into the tissue. The blood volume pulsations (ΔV) are related to both the systemic intravascular pulse pressure (ΔP) and the distensibility of the vascular wall (D) according to the relationship: $\Delta V = \Delta P \cdot D$.[27] The distensibility is influenced by intravascular volume status and sympathetic activity directed to the vessels, whereas vagal fibers are absent in the peripheral vascular bed. As a consequence, indices derived from the pulsatile PPGA are affected by a wide variety of stimuli exciting the sympathetic branch of the ANS. It is well known that nociception induces changes of ANS modulation toward a sympathetic activation.[24,28] Surgical Pleth Index, a PPGA-derived index, was proposed as a measure of nociception–antinociception balance at first[29,30]; afterward, it was demonstrated being affected by several confounding factors such as atropine administration, level of sedation, spinal anesthesia, intravascular volume status, and patient's position.[31–34] All these factors alter the ANS without affecting the nociception. We previously demonstrated that the changes of ANS modulation correlate with the changes of Surgical Pleth Index during general anesthesia.[35] We believe that this point is the focus of the misleading about pulse photoplethysmographic–derived indices as a measure of nociception–antinociception: In the present study, we provide the demonstration that pulse photoplethysmographic indices seem to reflect both sympathetic-mediated changes in vascular tone and sympathovagal efference to the heart.

Table 2. Correlations between Heart Rate Variability Parameters and Pulse Photoplethysmographic Indices

	PPGA	ANSS	ANSSi
μ_{RR}	0.24	0.474*	−0.551*
σ^2_{RR}	−0.178	−0.16	−0.073
HF_{RR}	−0.162	−0.113	−0.149
LF_{RR}/HF_{RR}	−0.225	−0.266	0.383†
μ_{SAP}	−0.209	−0.173	0.181
σ^2_{SAP}	0.271	0.123	0.142
LF_{SAP}	−0.307†	−0.35†	0.454*
αLF	0.042	0.088	−0.044
αHF	0.389†	0.54*	−0.667*

Values represent Spearman correlation coefficients.

* $P < 0.001$. † $P < 0.05$.

ANSS = autonomic nervous system state; ANSSi = autonomic nervous system state index; HF_{RR} = high-frequency spectral density of heart beat intervals; LF_{RR} = low-frequency spectral density of heart beat intervals; LF_{RR}/HF_{RR} = sympathovagal balance of the studied subjects; LF_{SAP} = low-frequency spectral density of systolic arterial pressure oscillations; PPGA = pulse photoplethysmographic amplitude; αHF = baroreflex sensitivity in the high-frequency band; αLF = baroreflex sensitivity in the low-frequency band; μ_{RR} = mean of heart beat-to-beat intervals; μ_{SAP} = mean of systolic arterial pressure; σ^2_{RR} = variance of heart beat-to-beat intervals; σ^2_{SAP} = variance of systolic arterial pressure.

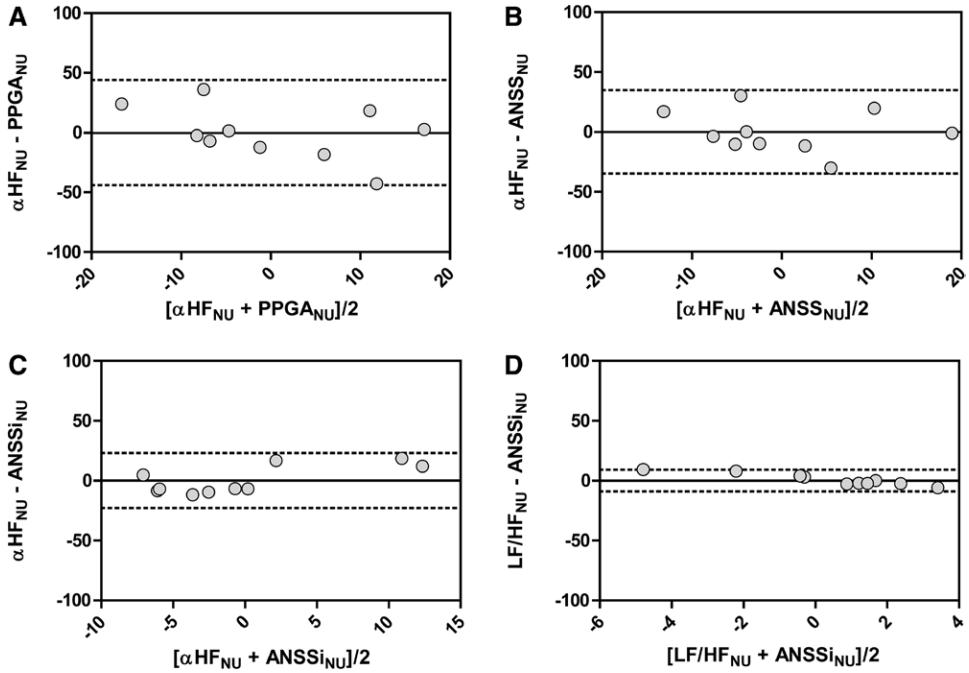

Fig. 3. Bland–Altman plot of agreement between couple of tested variables measuring autonomic nervous system modulation, corrected for within-subject variance with a linear mixed model. *Thick lines* represent the bias, and *dotted lines* represent ±1.96 SD. (*A*) αHF *versus* PPGA, bias = –0.04 (95% CI, –44.3 to 44.2); (*B*) αHF *versus* ANSS, bias = 0.09 (95% CI, –34.8 to 34.9); (*C*) αHF *versus* ANSSi, bias = 0.23 (95% CI, –22.7 to 23.2); (*D*) LF/HF *versus* ANSSi, bias = 0.96 (95% CI, –8.7 to 10.8). All data are given in normalized units. ANSS = autonomic nervous system state; ANSSi = autonomic nervous system state index; LF/HF = sympathovagal balance; PPGA = pulse photo-plethysmographic amplitude; αHF = baroreflex sensitivity in high-frequency band.

It is well known that passive HUT determines a pooling of blood toward the lower body accordingly to gravitational attraction and leads to a reduction of the venous return. These changes induce a reflected sympathetic activity and increase of muscle sympathetic nerve's firing rate aiming to squeeze the blood toward the upper body to maintain arterial pressure near to the level preceding the challenge.[9–13] Many human studies reported that increasing the angle tilt results in a greater muscle sympathetic nerve activity.[12,36–38] Because the sympathetic activation does not discriminate between upper and lower body, its effect is "massive" causing tachycardia, generalized vasoconstriction, and reducing blood flow even in the forearm.[26] We found that a greater intensity of sympathetic stimulation (at higher degree of tilt angle) did not changed significantly PPGA, ANSSi, and ANSS respect to HUT45. The reason of significance dearth between the two tilt angles might be due to a high activation of balancing sympathetic reflexes in response to the orthostatic challenge in healthy young subject since from lower degree angles. Furthermore, small differences between two different degrees of tilt could be detected with a large sample.

HRV analysis is a well-documented method to measure the sympathetic and vagal modulation directed to the heart and the vessels.[9–11] Conversely, some authors, in absence of adequate experimental validation, postulated that novel pulse photoplethysmograph–derived indices represent an adequate tool to measure ANS activity.[7,8] With this study, we provide an experimental evidence that pulse photoplethysmographic indices, especially ANSSi, increased in response to increases of sympathetic activity. To quantify the agreement between HRV and pulse photoplethysmograph–derived indices on measuring ANS modulation, we considered the ANS activity as an independent variable. Because the ANS activity is a concept that encompasses a dynamic sympathetic and vagal outflow (that is difficult to quantify as absolute size) and the analyzed variables have quite different measuring units, we normalize each series to better compare the variables with each others. We found that pulse photoplethysmograph–derived indices have a good agreement with the more validated HRV-derived indices as a measure of ANS modulation.

Methods for the HRV analysis in the frequency domain may be generally classified as nonparametric (*i.e.*, fast Fourier transform) and parametric (*i.e.*, autoregressive).[6] Although in most instances, both methods provide comparable results, in this study, we choose to analyze the heart rate and the systolic pressure variability with an autoregressive model. The advantages of this model are an easy identification of the central frequency of each component and an accurate estimation of frequency spectrum even on a small number of samples in which the signal is steady.[6] Moreover, autoregressive analysis provides a more reliable estimation of the HF component than the fast Fourier transform does.[39,40] Because the vagal efference detected in the HF band is synchronous

with respiratory activity, the healthy subjects in our studies breathed at a fixed respiratory rate of 0.3 Hz. This allows us to better measure the HF spectral component, because its central frequency was about in the middle of the HF band (0.15 to 0.5 Hz).

Our study has few limitations. We analyzed, from a physiologic point of view, the effects of sympathetic activity on pulse photoplethysmographic signals without administration of hypnotics or opiates. It has been demonstrated that general anesthesia reduces significantly ANS modulation on cardiovascular system.[41] Surgical stimuli can increase sympathetic outflow in these circumstances, but they introduce new variables in this research field: the intensity of surgical stimulation and the sickness of the studied patient. We strongly believe that a physiologic correlation between ANS activity and photoplethysmographic waves should be first assessed in a stable experimental condition in which all confounders should be reduced as much as possible. Furthermore, because the linear regressions showed highly skewed data, we applied a nonparametric correlation that ignores the impact of the nonindependence of observations that is created with the repeated measurements for each individual. This approach allows us to consider the crude association between the HRV parameters and the photoplethysmographic indices, but confounds within-subject variance and between-subject variance. These indices are only intended to serve as a gross measure of the association between the two measurements. The use of a mixed model (*i.e.*, with subjects having random effects) would be a large improvement in these circumstances. However, under most situations, this model would also be subject to extreme outliers.

In conclusion, in these healthy adults, we found that PPGA, ANSS, and ANSSi changed significantly in response to a sympathetic gravitational challenge. We believe that the results of this study provide a more extensive understanding of the physiologic basis of ANSSi: It seems to be a good and easy to keep surrogate to measure changes of the sympathovagal balance and sympathetic outflow directed to the vessels. HRV analysis provides a more comprehensive measure of ANS cardiovascular modulation taking in account each autonomic efference: (1) the vagal activity directed to the heart (HF_{RR}), (2) the sympathetic outflow directed to the vessels (LF_{SAP}), and (3) their interaction with the baroreflex control (αHF and baroreflex sensitivity in the LF band [αLF]). However, these measures are difficult to obtain in a clinical setting lacking automated, real-time calculation of these variables. Furthermore HRV analysis in the frequency domain requires the stationarity of the signals[6] often difficult to keep in a daily clinical practice. In this scenario, ANSSi might be a valuable tool displaying on anesthesia monitor the changes of global cardiovascular sympathetic modulation.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Colombo: Anestesia e Rianimazione 1, Azienda Ospedaliera "L.Sacco," Via G.B. Grassi 74, 20157 Milano, Italy. colombo.riccardo@hsacco.it.

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Appendix 1. Heart Rate and Systolic Arterial Pressure Variability Characteristics of the Studied Subjects

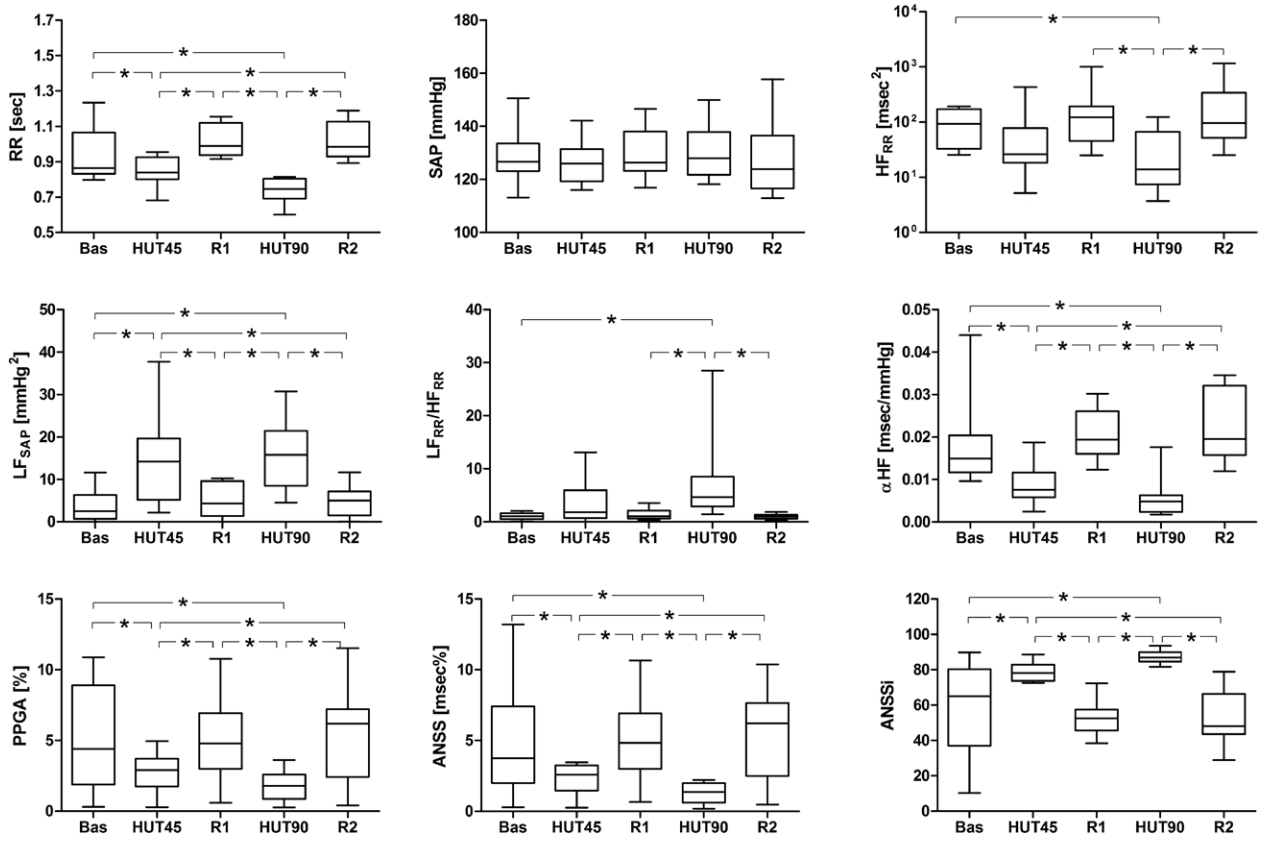
	Baseline	HUT45	R1	HUT90	R2	*P* Value (ANOVA)
μ_{RR} (ms)	863 (800–1,066)	838 (800–926)*	990 (937–1,121)	746 (691–805)*†	984 (930–1,127)	<0.0001
σ^2_{RR} (ms^2)	256 (173–343)	258 (132–504)	348 (260–503)	221 (116–362)	265 (189–585)	0.064
HF_{RR} (ms^2)	94 (32–172)	26 (18–78)	121 (46–194)	14 (7–66)*	96 (51–340)	0.025
LF_{RR}/HF_{RR}	1.08 (0.56–1.63)	1.86 (0.72–6)	1.04 (0.65–2.13)	4.66 (2.9–8.5)*	0.99 (0.63–1.37)	0.013
μ_{SAP} (mmHg)	127 (123–133)	126 (119–131)	126 (123–138)	128 (122–138)	124 (117–136)	0.64
σ^2_{SAP} (mmHg^2)	30.2 (21.5–42.4)	57.2 (21.2–63.7)	66 (29.8–78.5)	55.3 (30.7–67.6)	40.6 (21.9–54.7)	0.009
LF_{SAP} (mmHg^2)	2.5 (0.7–6.4)	14.2 (5.2–19.6)*	4.4 (1.4–9.6)	15.8 (8.5–21.4)*	5 (1.5–7.2)	<0.0001
αHF (ms/mmHg)	14.9 (11.7–20.4)	7.6 (5.7–11.6)*	19.4 (16.1–26.1)	4.8 (2.4–6.3)*	19.5 (15.8–32.2)	<0.0001
αLF (ms/mmHg)	9.4 (4.7–16.7)	9.1 (5–13.6)	13.9 (7.9–21.3)	7.2 (5.9–9.9)	16 (8.6–21.1)	0.11
PPGA	4.41 (1.89–8.9)	2.92 (1.75–3.71)*	4.79 (3.01–6.93)	1.8 (0.88–2.59)*	6.17 (2.41–7.21)	<0.0001
ANSS	3.74 (1.99–7.41)	2.57 (1.47–3.24)*	4.84 (3–6.91)	1.36 (0.61–1.98)*	6.21 (2.5–7.65)	<0.0001
ANSSi	64.9 (37–80.3)	78.3 (73.7–83)*	52.6 (45.7–57.6)	87 (84.6–89.9)*	48.1 (43.6–66.4)	<0.0001

Values are expressed as median (interquartile range). Values collected from the studied subjects at five time points during the study protocol. *P* values are assessed with one-way analysis of variance (one-way ANOVA). Significances between the study phases of μ_{RR} and αHF are simplified. In the table is reported only significance at HUT45 and HUT90. A more comprehensive significance of differences between all study phases is illustrated in appendix 2.

* $P < 0.05$ *vs*. baseline. † $P < 0.05$ *vs*. HUT45.

ANSS = autonomic nervous system state; ANSSi = autonomic nervous system state index; HF_{RR} = high-frequency spectral density of heart beat intervals; HUT45 = head-up tilt at 45°; HUT90 = head-up tilt at 90°; LF_{RR} = low-frequency spectral density of heart beat intervals; LF_{RR}/HF_{RR} = sympathovagal balance; LF_{SAP} = low-frequency spectral density of systolic arterial pressure oscillations; PPGA = pulse photoplethysmographic amplitude of the studied subjects; R1 = recovery after HUT45; R2 = recovery after HUT9; αLF = baroreflex sensitivity in the low-frequency band; μ_{RR} = mean of heart beat-to-beat intervals; μ_{SAP} = mean of systolic arterial pressure; σ^2_{RR} = variance of heart beat-to-beat intervals; σ^2_{SAP} = variance of systolic arterial pressure.

Appendix 2. Hemodynamic and Pulse Photoplethysmographic Variables during the Study Protocol



ANSS = autonomic nervous system state; ANNSi = autonomic nervous system state index; Bas = baseline; HF_{RR} = high-frequency spectral density of heart period; HUT45 = head-up tilt at 45°; HUT90 = head-up tilt at 90°; LF_{RR}/HF_{RR} = low frequency to high frequency ratio of heart period's spectral density; LF_{SAP} = low-frequency spectral density of systolic arterial pressure; PPGA = pulse photoplethysmographic amplitude; R1 = recovery after HUT45; R2 = recovery after HUT90; RR = heart period; SAP = systolic arterial pressure; αHF = baroreflex sensitivity in high-frequency band. * $P < 0.05$ between columns (*post hoc* Bonferroni test).